

## Rocks as museums of evolutionary processes

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### Abstract

The isolated granitic outcrops of Western Australia may harbour relics of the past such as *Isoetes* and *Stylidium merrallii*. They may also preserve a record of evolutionary change both within single populations and across population systems. Thus, the initial stages in the evolution of the complex hybrid genetic system are preserved amongst the extant lineages of *Isotoma petraea* on Pigeon Rock in the southwest of Western Australia. The elaboration of this bizarre genetic system is preserved across neighbouring outcrops. Study of *I. petraea* focuses attention on the evolutionary consequences of the controversial association of high levels of inbreeding with polymorphism for lethal genes within populations. In addition, it has generated novel and important insights into the genetic structure of Western Australian plant species and of evolutionary processes generally. Pigeon Rock and its unique living evolutionary museum is worthy of World Heritage listing, and should be preserved by removing the present stockyards and excluding the rock from the surrounding pastoral lease.

### Introduction

Granite rock outcrops may be regarded as evolutionary museums on a number of fronts. They may house relict flora, such as the primitive *Stylidium merrallii* (Kenneally & Lowrie 1994), *Isoetes*, an ancient gymnosperm, and relict fauna. Alternatively, they may provide windows on evolutionary processes. The species supported by the granite outcrop habitat necessarily exist in an archipelago of small populations. These populations have a genetic structure which is a consequence of the levels of communication (or gene flow) between them, and of the devices they have evolved to cope. This paper deals with the sequential consequences of isolation and inbreeding imposed on *Isotoma petraea* (Lobeliaceae) on these granite rocks by the species' habitat preference and evolved adaptations. It traces the evolution of the genetic system, from a conventional outbreeding state with normal chromosome behaviour and high levels of seed production, to complex hybridity with self fertilisation, highly restricted chromosome behaviour and stringent seed selection systems.

Classically, diploid sexual reproduction is thought to promote evolutionary capability by enhancing the level of genotypic diversity among gametes through recombination at meiosis and the distribution of that diversity between lineages by biparental reproduction (Fisher 1930; Müller 1932). In contrast, sex, comprising meiosis and fertilisation, is now widely recognised as having a more primitive role. Firstly, corruptions of the DNA molecule which escape biochemical correction through cellular DNA repair mechanisms may be corrected by recombinational processes, even in somatic cells (Bernstein *et al.* 1988). Secondly, mutations arising from errors in the repair processes or from mobile genetic element activity, and which have become incorporated into the genome, may be removed from

lineages by outcrossing and segregation (Bell 1988). While mutation is the ultimate source of the variation which is exploited in evolution, most mutations are deleterious, ranging from selectively neutral to lethal. Modern interpretations also suggest that diploidy has evolved as a redundancy (fail-safe) mechanism for protecting the soma against mutational corruption (Maynard Smith 1988). Providing it operates within an allelically diverse gene pool, sexual reproduction can also raise evolutionary capability to new levels relative to those achievable by mutation alone, and diploid gene pools are more capable than those associated with haploidy. Evolutionary capability is thus a consequence of unencumbered sexual genetic repair. If the sexual system is encumbered with devices which diminish its efficiency, both the evolutionary capability of the lineage and its ability to remove deleterious mutations are impaired.

Classical population genetic theory predicts that gene flow between populations comprising a species' metapopulation will bind it into a single evolutionary entity. Breakdown of this communication, by geographic isolation or by inbreeding, will lead to divergence of the non-communicating populations and, perhaps, speciation. Classical theory predicts that population divergence will be accelerated under conditions of inbreeding, leading to the fixation of alleles, including deleterious recessives, through genetic drift. It also indicates that genetic diversity can be maintained in diploid gene pools only when the heterozygote is the most fit genotype. This condition is readily achieved by associative overdominance in which loci may be rendered stably polymorphic by linkage to deleterious recessives (Zouros 1994), even in inbreeding populations. Thus, the occurrence of deleterious recessives, which may occur in large numbers and are often measurable in terms of lethal equivalents, may provide diploid sexuality, including inbreeding populations, with evolutionary advantage. However, these lethal equivalents result in a genetic load.

The expression of the genetic load in a diploid sexual

population will depend on the breeding system. In a normally outbreeding population, deleterious mutations are expected to be effectively invisible, being masked by the uncorrupted DNA of the homologous chromosome (diploid redundancy). Outbreeding populations are thus predicted to accumulate elevated levels of heterozygosity for deleterious mutations. If the population is forced to inbreed, this genetic load will be expressed as inbreeding depression due to the formation of elevated levels of deleterious recessive homozygotes. In a normally inbreeding population, on the other hand, mutations should be regularly exposed as homozygotes and purged out of the gene pool if they are sufficiently deleterious (Lande & Schemske 1985; Charlesworth *et al.* 1990). Thus inbreeders may be expected to be relatively free of seriously deleterious recessives, and exhibit little inbreeding depression and a reduced genetic load. Empirical evidence (Husband & Schemske 1996) suggests that in outbreeding plants, higher levels of inbreeding depression are exhibited at earlier stages in the life cycle, especially at seed formation, whereas with inbreeders, inbreeding depression is more likely to be expressed at later stages in the life cycle, if at all.

Genetic system evolution in *I. petraea* contradicts many classical expectations, particularly in its maintenance of high genetic loads in self-fertilising lineages. This paper reviews the genetic phenomena in *I. petraea* which led to this situation and documents the evolution, in response, of complex hybridity, a genetic system which effectively maintains genetic hybridity in highly inbred lineages. In addition, alterations to phenotypic characters, their patterns of variance within the population system, and their association with genetic system adaptations are discussed. Finally, the relevance of the observations made on *I. petraea* to patterns of variation in other native plant species is briefly reviewed.

### Inbreeding in *I. petraea*

*I. petraea* occurs in discrete isolated populations on granitic and other rocky outcrops of arid Australia. Due to this habitat preference, population size is often limited and subject to numerical fluctuation and genetic bottlenecks. These features alone would impose a measure of inbreeding upon the populations. In addition, populations may be characterised by different breeding systems. The primitive breeding system in *I. petraea*, and the Lobeliaceae generally, is one promoting outbreeding. Outcrossing may result when the stigma protrudes through the anther tube, dispensing the flower's pollen on the way, and then becomes receptive to pollen from other flowers.

Self pollination, or autogamy, results when the style fails to elongate and the stigma remains enclosed within the anther tube, receiving pollen from the same flower (James 1965). The frequency of stigma protrusion may be taken as a measure of outcrossing potential. Because of population size, habitat preference and the possibility of pollination between flowers on the same plant, populations exhibiting high frequencies of stigma protrusion may still be relatively inbred. In populations where little or no stigma protrusion occurs, inbreeding approaches 100 per cent. Thus, all *I. petraea* populations

are inbreeding to some extent, but some are much more inbreeding than others. The adoption of autogamy may be regarded as a means of reproductive assurance, and would be selected for where ovules would otherwise remain unfertilised (Jarne & Charlesworth 1993). In accordance with classical theory, inbreeding in *I. petraea* promotes genetic uniformity within and differentiation between populations.

### *I. petraea* Population Differentiation

*I. petraea* populations differ with respect to their stigma protrusion frequencies (Table 1). While stigma protrusion frequencies show some variation according to environmental conditions, the population differences are largely retained whether the plants are grown in the glasshouse, garden or in their natural habitat. This demonstrates that the stigma protrusion frequency is genetically determined, to a large extent, and that the populations are genetically differentiated with respect to this character.

Population divergence is also evidenced in flower colour and a number of metric characters such as leaf and flower dimensions, and the number of ovules per flower. Flower colour may vary from white, through varying shades of patterned pink to deep purple and blue. There is considerable variation between populations, and little within, so that populations are generally characterised by a single flower colour.

Partitioning of variance in leaf, pedicel and flower dimensions into within plant, within population and between population components was performed (James 1978; SH James, *unpublished data*). The characters measured were not closely related to aspects of plant fitness. The analysis (Fig 1; only structural homozygotes considered at this stage) showed that for these characters, while comparable variance components were found at all levels as would be predicted by classical genetics for

Table 1

Proportion of stigma emergence of plants from various populations of *Isotoma petraea*. Field measurements are given where possible. For plants from the same population, stigma emergence is usually higher in the more mesic garden and glasshouse environments than in the field. Measurements of stigma emergence other than those from the field will generally be an overestimate.

Population	Genetic System	Stigma emergence	Where Measured
Rainy Rocks	7II	1%	Field
Pigeon Rock	7II/⊙6	2%	Field
Iron Knob	7II	6%	Field
Victoria Rock	7II	32%	Garden
Merredin Peak	⊙14	40%	Garden
Boorabbin	7II	42%	Glasshouse
3 Mile Rock	7II/⊙10	56%	Garden
Moorine Rock	⊙12	79%	Garden
Bullabulling	7II	91%	Garden
Gnaribine Rock	7II	100%	Garden
Yacka Rock	7II	100%	Field
Disaster Rock	7II	100%	Field



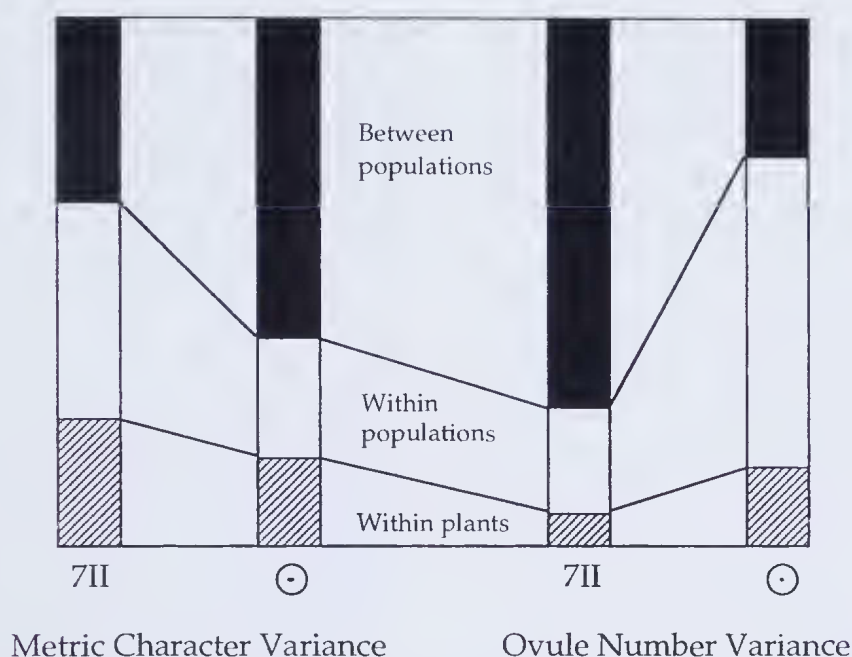


Figure 1. Partitioning of components of total variance for structural homozygote (7II) and complex hybrid (⊙) populations of *Isotoma petraea*. The distribution of variance reflects genetic system and selective importance of the characters (see text).

interbreeding populations, the greatest variance component was within populations. However, partitioning of variance for the average number of ovules per ovary (Kiew 1969) contrasted with that for the metric characters in that there was little variance within plants and by far the largest component was found at the between population level (Fig 1). This may be explained in that the number ovules produced may be expected to be a significant component of fitness and that it should be rigorously selected within populations. These observations generally conform with the expectation of differentiation between isolated populations.

### Inbreeding, the Distribution of Deleterious Alleles and the Accumulation of Lethals

Half of the gametes produced by an organism heterozygous for a deleterious mutation will be free of the defective allele. On selfing, one quarter of the progeny will be homozygous for the mutation, one half heterozygous, and one quarter free of the mutation. Thus, sexual reproduction allows a self-fertilising heterozygote to remove deleterious alleles. However, providing the deleterious homozygote is only slightly inferior to the heterozygote and the other homozygote, it may persist in the population (Charlesworth *et al.* 1990). With stochastic effects associated with small population size and bottlenecks, the deleterious allele may actually become fixed in the population. It then cannot be removed without genetic communication from another population. Further deleterious mutations may similarly become fixed leading to the accumulation of debilitating mutations by a process analogous to Müller's ratchet (Müller 1964; Lynch & Gabriel 1990).

In addition, if both of a pair of homologous chromosomes carry deleterious alleles, mutation-free

gametes can only be produced by a recombination event occurring between the two heterozygous loci at meiosis. In *I. petraea*, recombination processes, which result in chiasma formation at meiosis, are strongly localised to the ends of the chromosomes. This means that recessive deleterious alleles occurring in pairs in the body of each chromosome cannot be removed in a strictly self-fertilising lineage and will accumulate (James *et al.* 1990; James 1992). Normally in *I. petraea* there are 7 pairs of homologous chromosomes which form seven bivalents (7II) at meiosis. Each pair of homologous chromosomes is thus effectively a single supergenic locus with the two homologues being the two alleles (James *et al.* 1990; James 1992), each loaded with deleterious, even recessive lethal, mutations.

For each supergenic locus, 50% of the selfed products will be heterozygous and 50% homozygous, so for 7 such loci only  $1/2^7$  of the progeny will display parental levels of heterozygosity. The genetic load, that is, the proportion of inviable or inferior progeny of varying degrees of homozygosity for deleterious mutations, will approach 127/128 as the deleterious mutation content of each supergenic allele approaches lethality. This load strongly diminishes the reproductive potential of inbreeding populations, a problem which generates a focus for natural selection and adaptive evolution.

On the other hand, outbreeding populations handle their deleterious mutations much more effectively. Outcrossing facilitates the removal of deleterious alleles, even with chromosomes that show chiasma localisation. The lower frequency of homozygotes in outcrossing lineages, especially for newly mutated alleles, impedes any ratchet driven accumulation of deleterious alleles. The potential for genetic debilitation in populations with significant levels of cross pollination is therefore much lower or negligible, compared to that of strict inbreeders.

## Competitive Ability of 7II Material of Contrasting Levels of Hybridity

The debilitating effects of inbreeding in *I. petraea* have been demonstrated in several unpublished dissertations (Cohen 1982; Playford 1987; SH James & N Cohen unpublished data). Cohen (1982) compared the performance in competition experiments of selfed and outcrossed progenies from populations characterised by different levels of inbreeding. Pairwise competition trials were established by sowing, intermixed into pots, two progeny arrays of different origins (Table 2). Progeny arrays were derived from 7II selfs, 7II intrapopulational crosses, 7II interpopulational crosses and selfed complex hybrids (see below). Within pots there were generally a few clearly superior plants and a number of suppressed weaklings, and replicate pots were consistent. Almost all comparisons were highly determinate in their outcomes, with the superior genotype clearly apparent from both aerial biomass (Table 2) and number of plants. Where indeterminate results occurred, they were most likely in competitions involving progenies of the same hybridity class, and especially where interpopulational hybrids were involved. The mean competitive index calculable from the results may be viewed as a measure of the relative vigour of each progeny type.

The most effective competitors were the progeny of crosses within 7II populations, and by far the poorest

Table 2

Mean competitive index of progenies of differing hybridity classes, listed in decreasing order of competitive ability (see footnote).

Hybridity Class	Progeny Array	Mean Competitive Index
7II Intrapop Cross	7II Gnarlbine Rock	0.91
7II Self	7II Gnarlbine Rock	0.77
7II Intrapop Cross	7II Victoria Rock	0.82
7II Intrapop Cross	7II Boorabbin Rock	0.76
Complex Hybrid Self	The Humps	0.55
Complex Hybrid Self	Mt. Stirling	0.56
Complex Hybrid Self	Keokanie Rock	0.54
7II Interpop Cross	Victoria Rock x Boorabbin Rock	0.46
7II Interpop Cross	Yellowdine x Wargangering Rock	0.35
7II Interpop Cross	Victoria Rock x Mt. Caudan	0.11
7II Self	Boorabbin Rock	0.09
7II Self	Victoria Rock	0.01

Twenty five seeds of each of 2 progeny arrays of contrasting hybridity levels were sown intermixed and directly into a 125 mm pot containing a standard potting mix. Seeds had previously been tested to ensure uniform germination rates. All plants greater than 20 mm in height were then harvested at the soil surface after 6–8 months of free competition, counted, weighed and identified using GOT, LAP, PGM or IDH assays (James *et al.* 1983). This process was performed in triplicate for each pair of contesting progenies for 3 progeny arrays at each of 4 hybridity levels in all pairwise comparisons (198 pots). At harvest the relative aerial biomass of each competing type in each pot was calculated as a decimal fraction and averaged for each group of three replicates. The mean competitive index for each progeny type was calculated as the average of the aerial biomass decimals for all competitions involving the progeny array, but excluding within hybrid level comparisons. A similar index was calculated for relative numbers of plants; results closely mirrored those for aerial biomass and are not presented here.

were selfed progeny of the 7II plants (Table 2). An exception was the selfed 7II Gnarlbine Rock progeny, which was rated third best of all the progenies. These findings may be related to the degree of outbreeding in the source populations as indicated by their stigma protrusion frequencies. Both the Victoria Rock and Boorabbin populations have a reduced incidence of stigma protrusion (Table 1). These populations are composed of highly inbred lineages, each carrying their own suite of accumulated deleterious recessives, many of them in homozygous condition. Intrapopulational crosses will have many of these deleterious recessives masked in heterozygosity and thus display considerable heterosis over selfs. Gnarlbine Rock plants are much more outcrossed (Table 1), so that the lineages within this population may be expected to have accumulated fewer deleterious recessives. It is no surprise that self progeny from this population performed as well as intrapopulational crosses from other populations. They were, however, inferior to Gnarlbine Rock intrapopulational crosses, in keeping with expectations. The superiority in all cases (including Gnarlbine) of intrapopulational hybrid seedlings over selfed seed from the same population strongly suggests that competition amongst seedlings in natural seed beds would ensure the survival of only the most heterozygous types.

Intropopulational crosses were somewhat unpredictable and exhibited competitive abilities generally inferior to 7II intrapopulational crosses, but superior to 7II selfs (apart from the Gnarlbine Rock selfs; Table 2). Different arrays of deleterious mutations would have accumulated in the various genetically diverged and reproductively isolated 7II populations, so that crosses between them would be expected to result in heterotic hybrids. However, the unpredictable performance of the interpopulational cross progenies as compared to the deterministic performance of other hybridity classes indicates that these hybrids combine non-coadapted genomes, are somewhat unstable, and may be expected to exhibit less than optimal performance.

These results mirror previous work in which varying degrees of heterosis were generated in progeny of artificial interpopulational *I. petraea* hybrids (Beltran & James 1974). Dry weights of parental, F1 and F2 material, grown without competition under constant experimental conditions were compared. In all cases, the F1 yielded greater dry weight than the mid-parental value, and generally exhibited considerable heterosis over the poorer performing parent. In some cases the F1 performed better than both parents. These results conform to expectations when viewed in terms of stigma protrusion. The poorest performing parents (Iron Knob, Rainy Rock) had very low stigma protrusion frequencies (Table 1) and were highly inbred, so it was not surprising that F1s performed much better than the selfs of these parents. The most outbred populations in the study (Yackackine ["495"] and Disaster Rock) generally did not exhibit heterosis for dry weight accumulation when crossed to plants from other populations.

We conclude that inbreeding populations of 7II *I. petraea* are somewhat debilitated by the accumulation and fixation of deleterious recessive mutations and they show significant levels of heterosis following both inter-



and intrapopulation crossing. Inbreeding does not purge the deleterious recessives from the gene pool. In addition, a higher level of outbreeding in 7II *I. petraea* populations leads to the incorporation of only low levels of recessive lethals into the gene pool, a minimum genetic load and little, though definite, inbreeding depression. These conclusions refine the generally accepted relationships between the breeding system, genetic load and inbreeding depression and are best understood in terms of the role of sexuality in genetic repair or cleansing.

### The Pigeon Rock Population

The Pigeon Rock population is a very significant one in the evolution of *I. petraea* since it is in this population that complex hybridity evolved. Complex hybridity combines autogamy, a balanced lethal system and permanent hybridity for complexes generated by chromosomal interchanges (reciprocal translocations) (Darlington 1958; James 1965; Carson 1967; Holsinger & Ellstrand 1984). Pigeon Rock is composed of plants fitting two broad genetic types, with a number of lineages in each. Ancestral structural homozygote (7II) and derived complex hybrids co-exist in stable proportions of about 1:2 respectively (James *et al.* 1990).

Models for the evolution of the genetic system (James *et al.* 1990; James *et al.* 1991) propose that *I. petraea* on Pigeon Rock was initially 7II with an acquired autogamous habit (Table 1). This meant the population comprised essentially genetically isolated lineages, predisposed to the accumulation of deleterious mutations. The genetic load approached 127/128 as outlined above, with each supergenic allele carrying a lethal equivalent. In this genetic environment seed aborting lethals were generated, in the form of duplications and deficiencies, by chromosome segment

transposition, probably mediated by transposable elements. Abortion of seed due to these deficiencies had the benefit of reducing allocation of maternal resources into incompetent, homozygous seeds.

In Pigeon Rock complex hybrids, the initial seven supergenic loci have been reduced to five by coalescence of three of the bivalents into a single supergenic locus. During meiosis there are 4 bivalents plus a ring of six ( $\odot 6$ ) visible at metaphase 1. The  $\odot 6$  consists of 2 complexes, N and S, each of whose 3 chromosomes are placed alternately in the  $\odot 6$ , and held in place by chiasmata at their homologous ends. The duplications described above facilitated the origin of complex generating interchanges by enabling recombination between homologous segments in otherwise non-homologous chromosomes (James 1970; James *et al.* 1991).

Each  $\odot 6$  plant is an NS heterozygote which produces only N and S gametes. Gamete viability depends on regular segregation of the N and S chromosomes to opposite poles at anaphase-1 of meiosis. NN homozygotes are avoided since the N complex is not transmissible in pollen. SS homozygotes are eliminated by seed abortion, elaborated from the seed abortion system which existed prior to complex hybridity (James *et al.* 1991). The NS  $\odot 6$  complex hybrid is superior to the 7II structural homozygote in that it potentially represents a 4-fold increase in the number of fully heterozygous progeny ( $1/2^5$  compared to  $1/2^7$ ). The genetic load is thus reduced to 31/32.

The proposed model for the evolution of complex hybridity is supported by a number of lines of evidence. Firstly, mathematical models suggest that, given the constraints seen at Pigeon Rock, complex hybridity will rise to the level of prominence seen there (James *et al.* 1990). Secondly, allozyme marker heterozygosity, which would also act as a marker for deleterious mutation

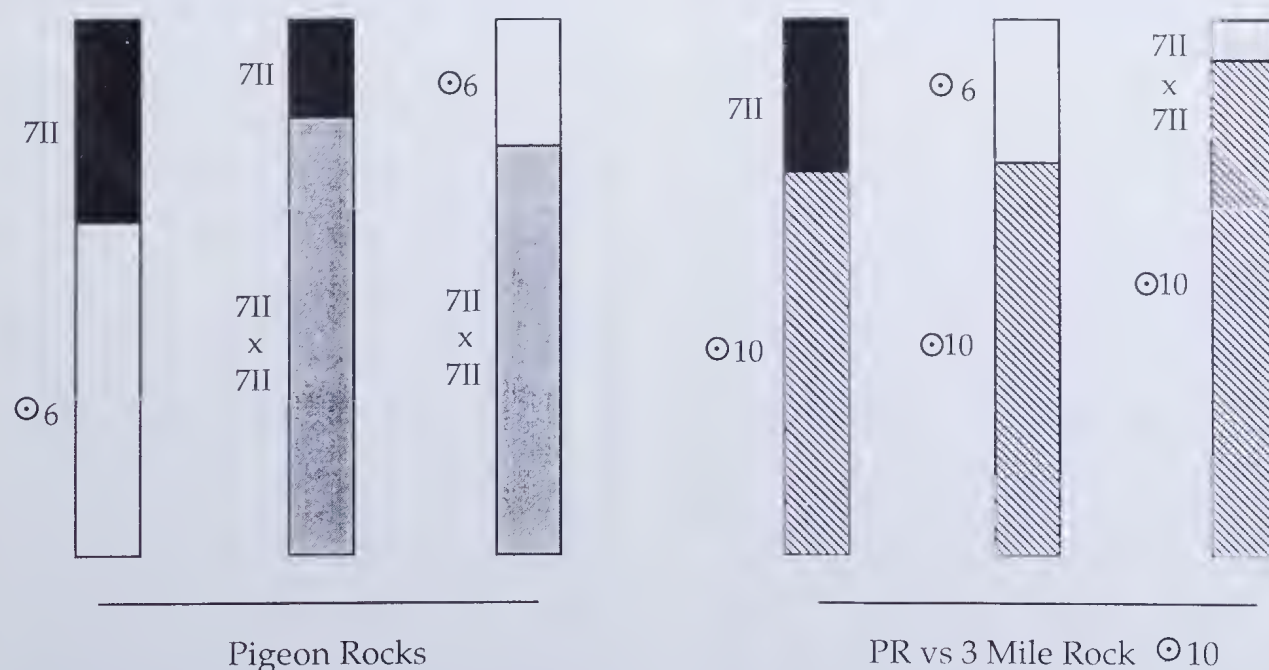


Figure 2. Competition experiments involving Pigeon Rocks selfed 7II, intrapopulation 7II x 7II crossed, selfed 6 and selfed 3 Mile Rock 10 progenies. Methods similar to those described in Table 2.

heterozygosity, is noticeably greater in the  $\odot 6$  than the 7II (James *et al.* 1983). Thirdly, a series of competition experiments (Playford 1987) similar to those described above showed that the various levels of hybridity at Pigeon Rock were rated as might be expected from Table 2. Selfed 7II material from Pigeon Rock performed extremely poorly when pitted against its intrapopulation 7II  $\times$  7II rivals (Fig 2). The selfed 7II was inferior almost to the same extent against the selfed  $\odot 6$  (Fig 2), a finding of obvious importance for the establishment and maintenance of the genetic system at Pigeon Rock. 7II  $\times$  7II crosses were superior to the  $\odot 6$ , but these would occur only very rarely, if at all, in the natural population and would not appreciably affect the 7II: $\odot 6$  balance. Finally, preliminary results of a molecular phylogeny using Random Amplified Polymorphic DNA strongly support the hypothesis that the  $\odot 6$  originated from a Pigeon Rock 7II lineage exhibiting elevated seed abortion.

Resolution of the mechanism of evolution of complex hybridity on Pigeon Rock is particularly instructive. It demonstrates circumstances in which recessive lethal genes which operate early in seed development may accumulate in inbred lineages, a somewhat unexpected and controversial phenomenon (*cf* Husband & Schemske 1996).

### Elaboration of Larger Ringed Complex Hybrids

There are many populations of complex hybrid *I. petraea*, all extending in a south-westerly direction from Pigeon Rock (James 1965; James 1970). It is thought that a series of migrations occurred, starting with the Pigeon Rock  $\odot 6$ . Hybridisation of an immigrant  $\odot 6$  complex hybrid from Pigeon Rock with plants of the invaded 7II population would generate heterotic hybrids which were also heterozygous for numerous duplications and deficiencies arising from the seed abortion systems in the Pigeon Rock parent. The duplications facilitated further interchanges and allowed the coalescence of the genome into fewer supergenic loci, while the deficiencies formed the basis of a new balanced lethal system. Thus, interpopulation hybridisation involving the Pigeon Rock  $\odot 6$  complex hybrid was able to generate new complex hybrids with larger rings in the recipient population. Migration of the new, larger ringed complex hybrid to the next 7II population led to further elaboration of the complexes so that the number of chromosomes incorporated into the ring progressively increased, in general, the further from Pigeon Rock. Usually each population is characterised by a single meiotic configuration and a very restricted array of genotypes (James 1970; James *et al.* 1983), indicating that a single, most fit complex hybrid type has displaced all others. Complex hybrid populations at the extreme of the genetic system's range are  $\odot 14$ . The  $\odot 14$ s have a single supergenic locus and a genetic load reduced to 1/2.

Competition experiments have again demonstrated the feasibility of this model. Complex hybrids performed somewhat better than interpopulation 7II  $\times$  7II crosses, and markedly better than 7II selfs (Table 2). The 3 Mile Rocks  $\odot 10$  out competed both 7II and  $\odot 6$  material from Pigeon Rock (Fig 2) indicating that, at least in this case,

the larger ring confers more vigour than the smaller one. The  $\odot 10$  was also victorious over Pigeon Rock 7II intrapopulation crosses (Table 2), which was perhaps not according to expectations, but which might be explained on the grounds of a generally high level of relatedness and genetic debilitation among the Pigeon Rock 7IIs.

Complex hybridity is a highly specialised system and very stable once established in a population. Crosses between plants from different complex hybrid populations display negative heterosis in the F1 (Beltran & James 1974), suggesting that a level of hybrid vigour or heterosis at least equivalent to that exhibited by 7II  $\times$  7II interpopulation crosses (see Table 2) is already conserved within plants. In addition, it may be expected that the two complexes associated in a naturally occurring complex hybrid are highly selected for mutual co-adaptation whereas complexes from different populations, which have not been mutually selected, are not coadapted and perform less well together.

The selective advantage of complex hybridity has been explained, in the past, in terms of a "pursuit of hybridity" (Darlington 1939; James 1965; Carson 1967). Detailed study of the genetic properties and competitive abilities of natural populations of *I. petraea* distributed over the granite rocks of Western Australia has led to identification of the masking of deleterious mutations as the underlying basis of hybrid superiority and to a detailed appreciation of the pathways followed in this evolutionary pursuit. CD Darlington's concept of the "pursuit of hybridity" in evolutionary progress has always been controversial, but we can be certain that it is a real and powerful phenomenon.

### Adaptive Responses of Complex Hybrids

The pursuit of hybridity in highly inbreeding populations of *I. petraea* has led to the evolution of complex hybridity. In this genetic system, the seven pairs of chromosomes have been coalesced to form two complexes which are mutually coadapted alleles of a single supergene. These complexes are distributed from parent to offspring without change, for there is essentially no recombination within their chromosomal components, and their components cannot assort independently as is possible in the primitive seven bivalent forming types. Thus, recombinational capability, which is often taken to be the primary value of sexual reproduction in normal diploid sexual species, and the source of evolutionary capability, is highly suppressed in these derived complex hybrids. However, the complex hybrids exhibit quite striking evolutionary adaptations in important components of their reproductive biology (summarised in Table 3).

First, the complexes for which the complex hybrids are heterozygous increase in size, from three chromosomes in the Pigeon Rock  $\odot 6$ , through to seven in the  $\odot 14$ s. The changes, from outbreeding to inbreeding through to the adoption of complex hybridity and increasing ring size, are mirrored by corresponding changes in heterozygosity, genetic debilitation and competitive ability (Table 3).



Table 3

Summary of *Isotoma petraea* breeding system and morphological adaptive responses to changes in genetic system.

	Primitive Forms		Pigeon Rock		Complex Hybrids
Cytology	7II	7II	7II	⊙6	⊙10-⊙14
Stigma Protrusion	High	Low	Very Low	Very Low	Variable
Observed Heterozygosity	Low	Low	Low	Medium	High
Competitive Ability	High	Low	Low	Medium	High
Debilitation	Low	High	High	Low?	None Expected
Interpopulational Heterosis	Low	High	n/a	n/a	Negative
Hybrid Instability	Yes	Yes	?	?	Yes
Flower Size	Large	Large	Large	Medium	Small
Ovule Number	Low	Low	Low	Low	High
Seed Aborting Lethals	Low	Low	Low-Medium	Medium-High	High, Early

Second, complex hybrid plants are characterised by a smaller flower size than 7IIs (James 1982a), a trend evidenced even within the Pigeon Rock population where there is a distinct floral dimorphism dependent on the genetic system (Table 3). A reduction in flower size is commonly associated with autogamic pollination (Jarne & Charlesworth 1993) and presumably represents the loss of a function (attraction of pollinating vectors) where such a function is irrelevant.

Third, the accumulation of chromosomes into interchange rings in complex hybrids results in meiotic irregularities and reduced gametic fertility (Darlington 1958; James 1970; Cleland 1972). Gamete fertility, 100% in 7II forms, is reduced to about 70% in ⊙6s and about 20% in ⊙14s. Fully half of the selfed progeny from the remaining gametes would be homozygous for the complexes and therefore not viable, so the potential fecundity of the ⊙14s is about 10% that of the 7IIs. Despite this loss of fecundity, the proportion of fully heterozygous progeny in complex heterozygotes is still higher for all ring sizes than for 7IIs (James 1970). The loss of fecundity is compensated for, in complex heterozygotes, by a substantial increase in ovule numbers (Table 3). A study of seven 7II and sixteen complex hybrid populations (Kiew 1969) showed that the 7II plants had an average of 1130 ovules per flower, while the complex hybrids averaged 1545. The Pigeon Rock ⊙6 had an average ovule number of 1090, a figure close to the average for the 7II populations, and to that for alethal Pigeon Rock 7IIs (JD Bussell unpublished data).

Fourth, the complex homozygote elimination system shows significant evolutionary development amongst *I. petraea* complex hybrids (Lavery & James 1987). The recessive seed aborting lethals which removed the SS complex homozygotes in the Pigeon Rock ⊙6s were relatively late acting so that the aborted seed exhibited a well developed but collapsed testa. As rings become larger, and as distance from Pigeon Rock increases, the time of seed abortion becomes earlier and earlier, so that in some large ringed forms, the aborted seeds are barely distinguishable from unfertilised ovules (Table 3). Earlier seed abortion allows fewer resources to be invested into incompetent seed. In some populations, homozygote elimination is based on complementary gametic lethality so that no resources are invested into incompetent complex homozygotes.

Distribution and Generation of Variation

The profound genetic system change, from relatively open sexuality to essentially invariant complex hybridity, might well be expected to be associated with a redistribution of variation within the population system (e.g. Darlington 1958; Jarne & Charlesworth 1993). This has been studied by examining the distribution of phenotypic variation within and between populations of *I. petraea* (Kiew 1969; SH James unpublished data). Patterns of variation were found to be dependent on the genetic system and on the selective importance of the character under examination, as detailed below.

The distribution of variance for ovule number, a character directly linked to the reproductive capability and fitness of plants, is strikingly different in the 7IIs and complex hybrids (Fig 1). In the 7IIs, most variance for ovule number occurs between populations, there is little between plants and very little between flowers within plants. Amongst the complex hybrids, on the other hand, most variance for ovule number occurs between plants within populations – the level at which selection occurs. Complex hybrids also have a significant amount of variation within plants. The complex hybrids' uniformly higher ovule number suggests that directional selection for ovule number is very strong in complex hybrid populations and less so among the 7IIs. Presumably this elevated ovule number is a response to the considerably reduced probability of an ovule becoming a seed, as described earlier.

In contrast, for metric characters (including leaf shape and the length of the peduncle) which have no obvious bearing on reproductive capability, the variance within the primitive 7IIs is distributed almost equally between populations, between plants within populations, and within plants (Fig 1). Amongst the derived complex hybrids, however, the variance between populations is relatively larger, and the variance between plants within populations, and within plants, is reduced.

The expression of phenotypic attributes is a complex process determined by the interaction of many genes. Characters which show constant expression, and therefore low levels of within plant variation, are said to exhibit a high degree of canalization, or homeostasis. Characters exhibiting higher levels of within plant variation are determined by less well canalized gene systems. Evolutionary change of a homeostatic character

can only be achieved when the canalizing gene systems are destabilised so that selectable variation becomes exposed in the population.

In general, heterozygous genotypes tend to exhibit more homeostatic control than homozygous genotypes (e.g. Mitton & Grant 1984; Palmer & Strobeck 1986; Jarne & Charlesworth 1993). However, wide crosses, as well as inbreeding, may lead to the destabilisation of developmental processes (Clarke 1994; Freeman *et al.* 1994). These observations may explain how the complex hybrids, with generally optimum levels of enforced heterozygosity, have a relatively lower within plant variance for metric characters than the inbreeding 71ls (which are more homozygous for a range of deleterious mutations), and yet have a higher proportion of within plant variance for ovule number than the 71ls (Figure 1).

In any population there is a fine balance between rigid control of developmental processes and maintenance of adaptive capacity. *I. petraea* offers a superb resource for studies into the effect of varying degrees of inbreeding and homozygosity on developmental processes and phenotypic variability in plants. Despite abandonment of recombination often being described as an evolutionary dead end (Darlington 1958; Carson 1967; Wagner & Gabriel 1990), important selectable diversity may still be generated in *I. petraea*, even in complex hybrids. Perhaps, along with hybridisation and the vestiges of sexual recombination and segregation, other more primitive mechanisms are utilised for genetic diversification and genome reorganisation. In particular, mobile genetic element activity destabilises the genome, it may affect particular parts of the genome more than others, and it may be promoted by "genomic shock", where mutually uncoadapted genomes are combined in wide crosses.

## Conclusions

The network of granite rocks characterising large areas of the Australian continent are of particular importance as museums for relict flora, fauna, and of evolutionary processes. They provide a living collection of discrete yet integrated natural evolutionary experiments which generally so far have been sampled in only the most cursory fashion. Resolution of the evolutionary pathway leading to complex hybridity in *I. petraea* has provided a unique perspective on the consequences of inbreeding. Competition experiments reported herein, and previous work (Beltran & James 1974), have demonstrated distinct competitive advantages at critical stages in the proposed evolutionary pathway. At a more fundamental level, the *I. petraea* story supports the theory that biparental sexual reproduction and meiotic recombination are primarily concerned with genetic repair. Impediments to these processes result in the accumulation of deleterious mutations, genetic load, inbreeding depression and debilitation of whole populations. Importantly, and contrary to classical expectations, inbreeding, and especially intense inbreeding, does not purge the genetic load. Load in *I. petraea* may be managed either by outbreeding or, in inbreeding populations, by the early elimination of incompetent homozygotes and strict control of recombination via complex hybridity.

Interpopulational hybrids and the naturally occurring complex hybrids demonstrate considerable variation (decanalization) for morphological and reproductive characteristics and for competitive ability. These hybrids are comparable in many ways to the mutator genotypes generated by interracial hybrids of *Drosophila* (Thompson & Woodruff 1978), some of which induce mobile genetic element activity (Fontdevila 1988). It seems likely that mutator genotypes, generated by interfacing separately co-adapted gene pools, release an innovative capability which is more primitive than the genetic innovation associated with diploid sexuality, and which has been, and still is, of profound importance in organic evolution. This harks back to Darlington (1956) who, in discussing the nature of variation in natural systems, concluded that the genome's ability to generate biological capability and variability must be more important in enabling biological evolution than the environmental factors promoting it. The possible role of mobile genetic elements in mutator genotype activity in *I. petraea* remains to be investigated.

It is hoped that this paper has presented a picture of the granite rocks scattered throughout arid Australia, not so much as self-contained inselbergs to be managed and conserved as isolated units, but as networking subdivisions of species' metapopulations and as places of active, documentable evolutionary change. They are ideally suited, as museums of evolutionary processes, for the study of genetic behaviour, variation and evolutionary responses. *I. petraea* has proved to be most illuminating in the study of these phenomena, and will no doubt continue to be so.

*I. petraea* has been instrumental in the development of an understanding of the presence and action of seed aborting lethal genes in many of Western Australia's signature floral genera, including *Stylidium*, *Anigozanthos*, *Drosera*, *Laxmannia* and *Eucalyptus* (reviewed by James 1982a,b, 1992, 1996; Burbidge & James 1991). Recently, Kennington & James (1997) have outlined a mechanism whereby recessive lethal and mildly deleterious genes may interact to prevent purging of load in eucalypt species. Study of *I. petraea* has also helped elucidate notions of genomic coalescence as a potent evolutionary force (reviewed by James 1992). Pigeon Rock, set in the midst of superb, relatively undisturbed country, is of considerable importance as the putative origin of complex hybridity in *I. petraea*. This inselberg warrants special conservation status, including removal of the present stockyards, exclusion from the surrounding pastoral lease and preclusion of vermin. In fact, we feel that Pigeon Rock warrants World Heritage listing.

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